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Docket Number: UPN-4105 (M2351) Title: Compositions and Methods of Using Capsid Protein from Flaviviruses and Pestiviruses Inventors: David B. Weiner and Joo-Sung Yang Sheet 1 of 30

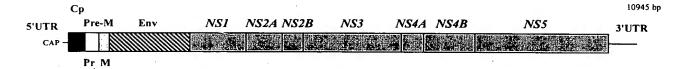
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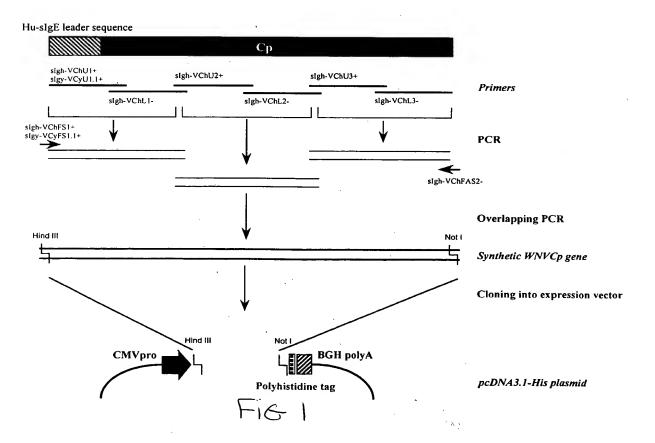
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Cloning Strategy for WNV-HNY1999 Capsid Gene: pWNVh-DJY, pWNVy-DJY



Docket Number: UPN-4105 (M2351)
Title: Compositions and Methods of Using Capsid Protein from Flaviviruses and Pestiviruses Inventors: David B. Weiner and Joo-Sung Yal Sheet 2 of 30

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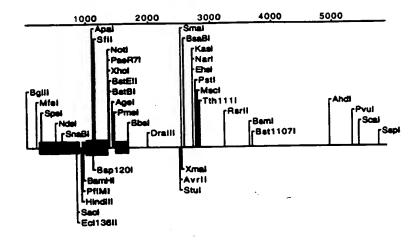


Fig. 2

Docket Number: UPN-4105 (M2351)
Title: Compositions and Methods of Using Capaid Protein from Flaviviruses and Pestiviruses Inventors: David B. Weiner and Joo-Sung Yan

Tuesday, September 5, 2000

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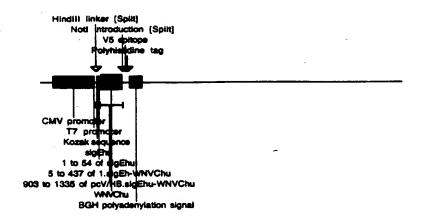
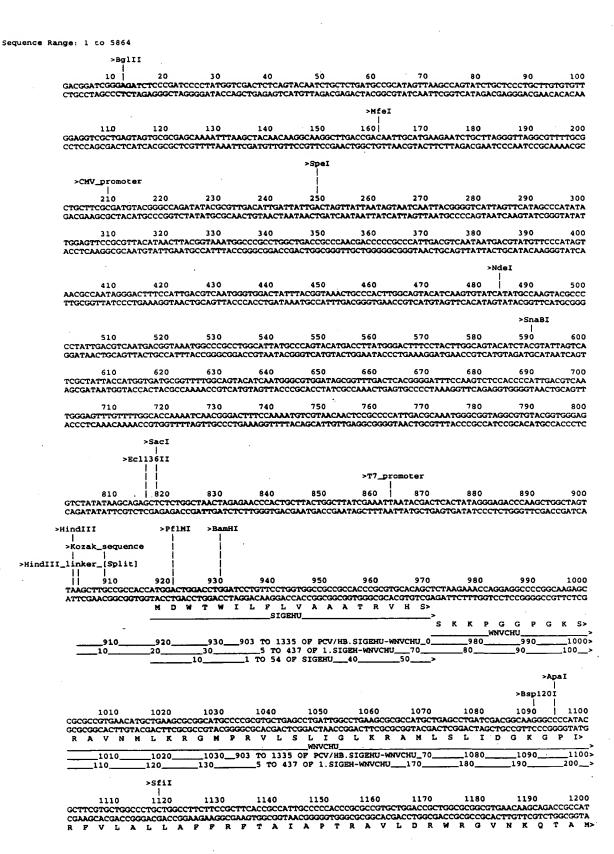


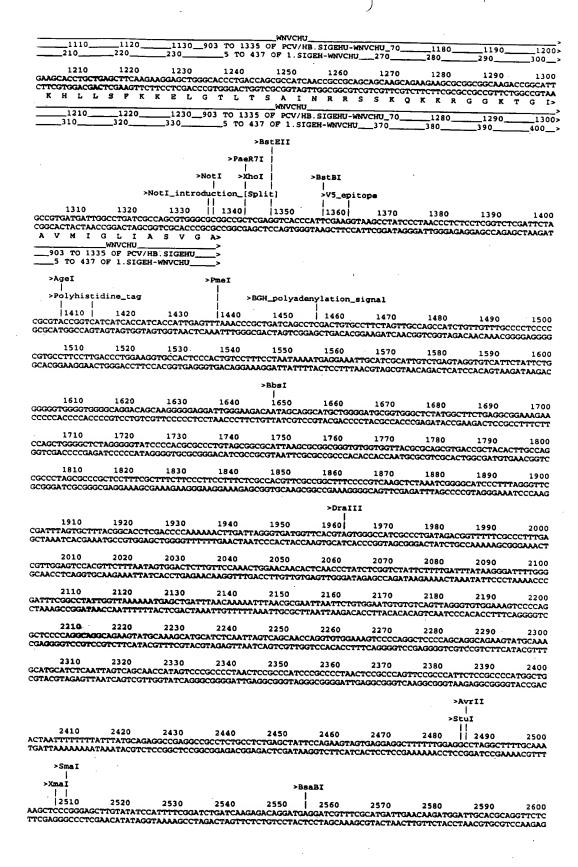
Fig. 3

Docket Number: UPN-4105 (M2351)
Title: Compositions and Methods of Using Capsid
Protein from Flaviviruses and Pestiviruses
Inventors: David B. Weiner and Joo-Sung Ya
Sheet 4 of 30

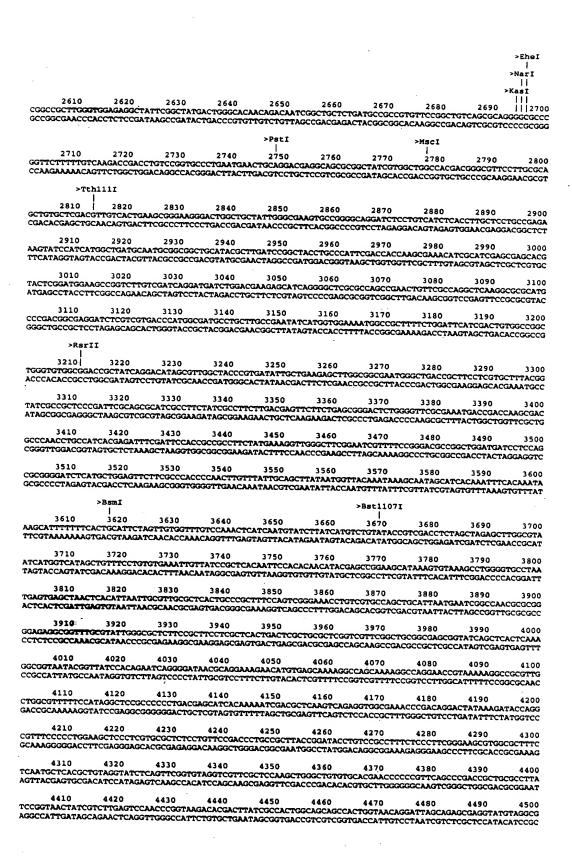




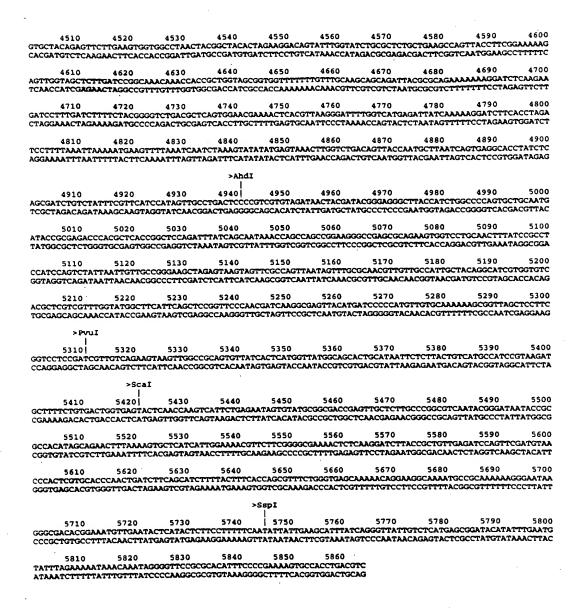
Docket Number: UPN-4105 (M2351)
Title: Compositions and Methods of Using Capsid rotein from Flaviviruses and Pestiviruses ventors: David B. Weiner and Joo-Sung Yang Sheet 5 of 30

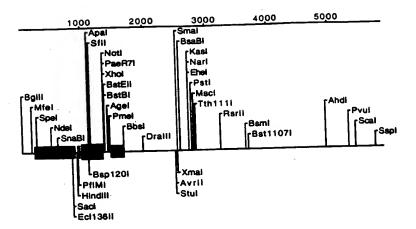


Docket Number: UPN-4105 (M2351)
Title: Compositions and Methods of Using Capsid
Protein from Flaviviruses and Pestiviruses
Inventors: David B. Weiner and Joo-Sung Yang
Sheet 6 of 30



Docket Number: UPN-4105 (M2351)
Title: Compositions and Methods of Using Capsid Protein from Flaviviruses and Pestiviruses Inventors: David B. Weiner and Joo-Sung Yang Sheet 7 of 30





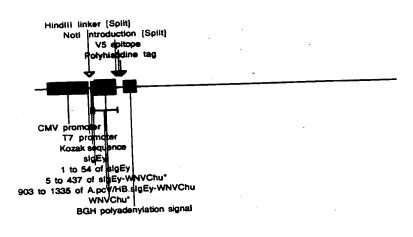
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Docket Number: UPN-4105 (M2351)
Title: Compositions and Methods of Using Capsid tein from Flaviviruses and Pestiviruses ventors: David B. Weiner and Joo-Sung Yang Sheet 9 of 30

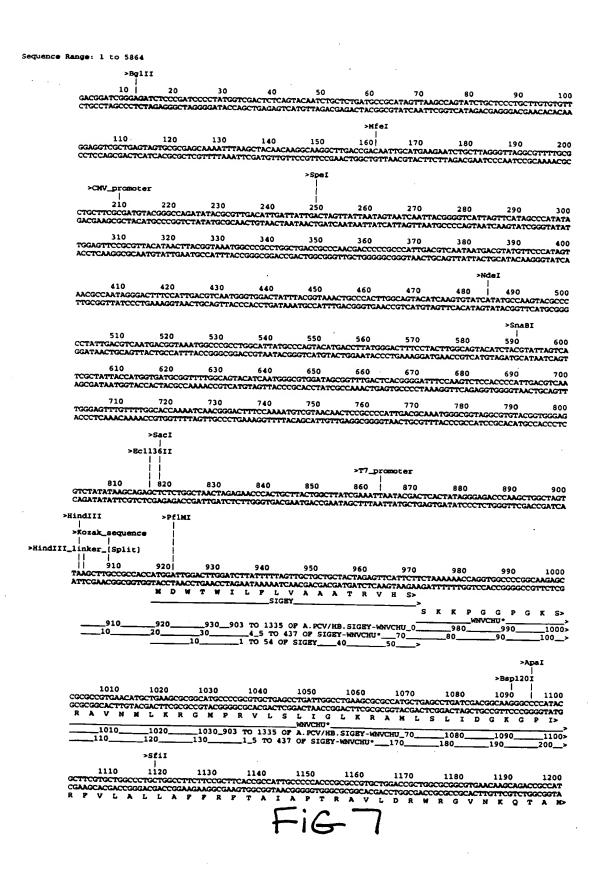


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FIG. 6

Docket Number: UPN-4105 (M2351)
Title: Compositions and Methods of Using Capsid
Protein from Flaviviruses and Pestiviruses
Inventors: David B. Weiner and Joo-Sung Yang
Sheet 10 of 30

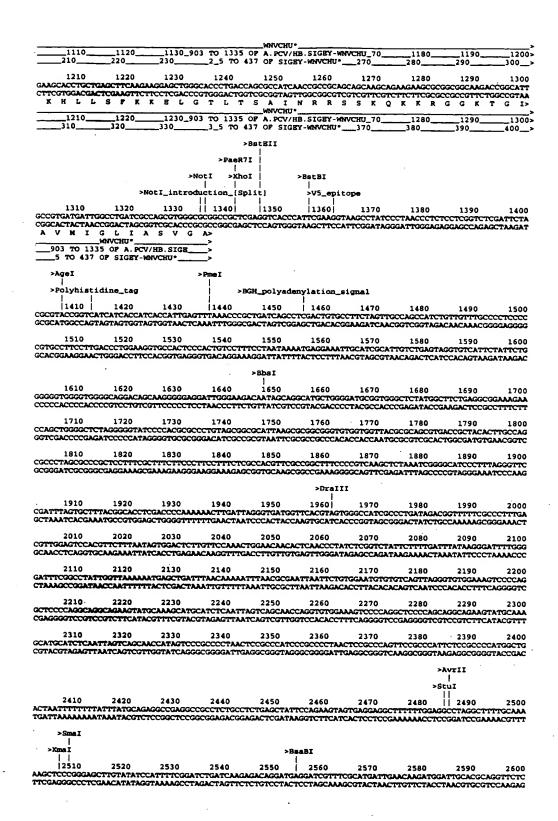




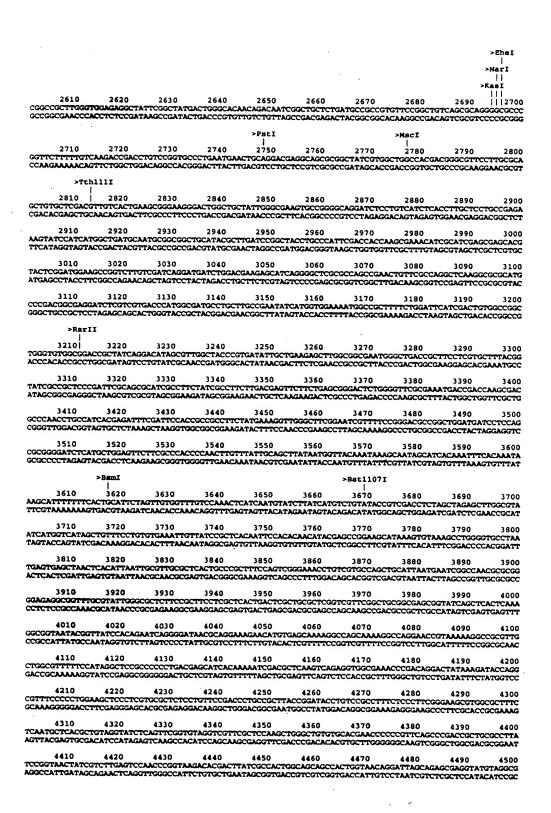
Docket Number: UPN-4105 (M2351)
Title: Compositions and Methods of Using Capsi
Protein from Flaviviruses and Pestiviruses
Inventors: David B. Weiner and Joo-Sung Yang
Sheet 11 of 30

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ACGCTCGTTTGGTTTGGTTTCATTCACTCCGGTTCCAACGATCAACGCGAGTTACATCATCATCCCCATGTTTTGCAAAAAAACGCGTTAGTCCTTC TOCGAGCAGCAAACCAACCAACCTACTCCAATGTACTCCGGTTCATTTCCGCTCAATGTACTCCCCAATGTACTCCCAATCCAACACCGTTTTTTCCCCAATCCAACACCGTTTTTTCCCCAATCCAACACGAGAAG	GGTAGGTCAGATA	NTTAACAACG	CCCTTCGAT	TCATTCATCA	LAGCGGTCAA	TTATCAAACG	CGTTGCAACA	ACGGTAACGA	TGTCCGTAGC	ACCACAG
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CCCACTCGTGCACCCAACTGATCTTCAGCATCTTTTACTTTCACCAGCGTTTCTGGTGAGCAAAAACAGGAAGGCAAAATGCCGCAAAAAAGGGAATAA GGGTGAGCACGAGGTTGACTAGAAGTCGTAGAAAATGAAGTGGTCGCAAAGACCCACTCGTTTTTGCCTTCCGTTTTACGGCGTTTTTCCCTTATT SSBJI 5710 5720 5730 5740 5750 5760 5770 5780 5790 5800 GGGCGACACGGAAATGTTGAATACTCATACTCTTTCTTTTTCAATATTATTTGAAGCATTTTATCAGGGTTATTGCTCCATGAGCGGATACATATTTGAATG CCCGCTGTGCCTTTACAACTTATGAGTAATGAGAAGGAAAAAGTTATAAACTTAC 5810 5820 5830 5840 585 5860 TATTTAGAAAAATAAACAAATAGGGGTTCCGCGCACATTTCCCCCGAAAAGTGCCACCTGACGTC										
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TATTTAGAAAATAAACAAATAGGGGTTCCGCGCACATTTCCCCGAAAAGTGCCACCTGACGTC	5810	5820	5830	5840	5850	5860				
	TATTTAGAAAAAT	AAACAAATAG	GGGTTCCGCG	CACATTTCCC	CGAAAAGTGC	CACCTGACGT				

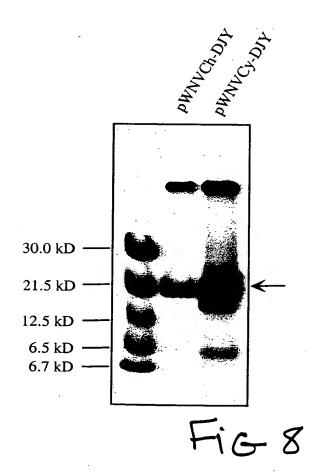
Docket Number: UPN-4105 (M2351)
Title: Compositions and Methods of Using Capsid rotein from Flaviviruses and Pestiviruses ventors: David B. Weiner and Joo-Sung Yang Sheet 12 of 30



Docket Number: UPN-4105 (M2351)
Title: Compositions and Methods of Using Capsid
Protein from Flaviviruses and Pestiviruses
Inventors: David B. Weiner and Joo-Sung Yang
Sheet 13 of 30



35S-Labelled in vitro Translated Products of pWNVCh-DJY and pWNVCy-DJY



WNV Capsid (Cp) Peptides - Location and Sequences

	e.	
120	MIGLIASVO	
110	KRGGKTGIAV	KRGGKTGI -P3
100	AINRRSSKQK	LTSAINRRSSKQKKRGGKTGI WNVC-P3
06	FKKELGTLTS	TLTS
80	OTAMKHLLSE	
70	VLDRWRGVNK	
09	RFTAJAPTRA	
20	RFVLALLAFF	RFVLA 12
40	LSLIDGKGPI	KRAMLSLIDGKGPIRFVLA WNVC-P2
30	SLIGLKRAM	KRAM
50	MLKRGMPRVI	IMLKRGMPR 1
10	MSKKPGGPGKSRAVNMLKRGMPRVLSLIGLKRAMLSLIDGKGPIRFVLALLAFFRFTAIAPTRAVLDRWRGVNKQTAMKHLLSFKKELGTLTSAINRRSSKQKKRGGKTGIAVMIGLIASVGA	SKKPGGPGKSRAVNMLKRGMPR WNVC-P1
WNV Cp	Sequence	Peptide Seq. Peptide Name

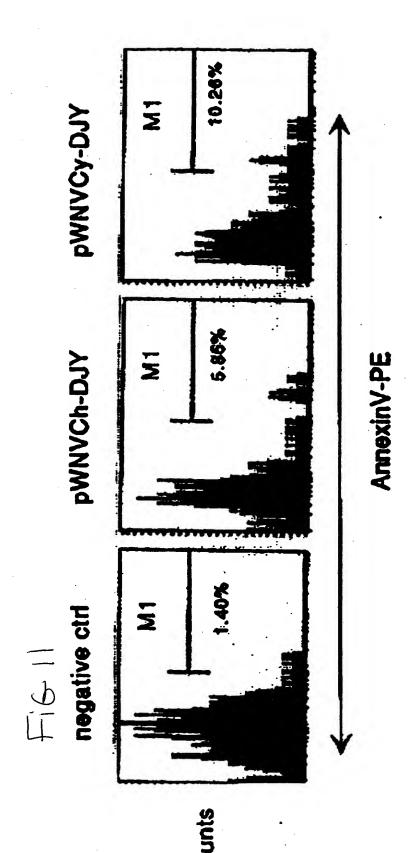
Fig.9

Docket Number: UPN-4105 (M2351)
Title: Compositions and Methods of Using Caps
Protein from Flaviviruses and Pestiviruses
Inventors: David B. Weiner and Joo-Sung Yang
Sheet 16 of 30

pcDNA3+protein pWNVQ+no Ag pWNVQ+protein pWNVQ+protein Q450 IFFAPC pcDNA3+no Ag IFIN-APC **9**

Fig. 1

Docket Number: UPN-4105 (M2351)
Title: Compositions and Methods of Using Cap
Protein from Flaviviruses and Pestiviruses
Inventors: David B. Weiner and Joo-Sung Yang
Sheet 17 of 30



Docket Number: UPN-4105 (M2351)
Title: Compositions and Methods of Using Capsid otein from Flaviviruses and Pestiviruses ventors: David B. Weiner and Joo-Sung Yang Sheet 18 of 30

Fig. 12A

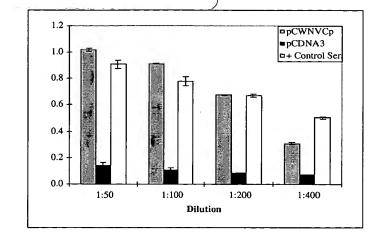


Fig. 12B

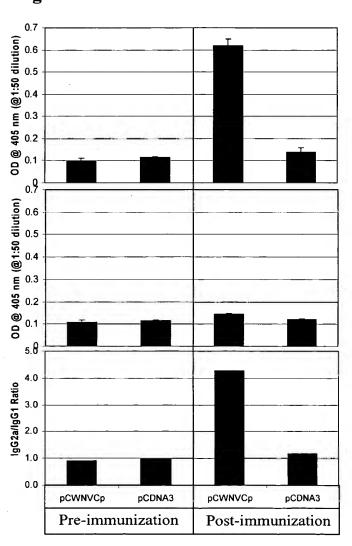
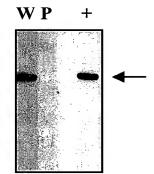
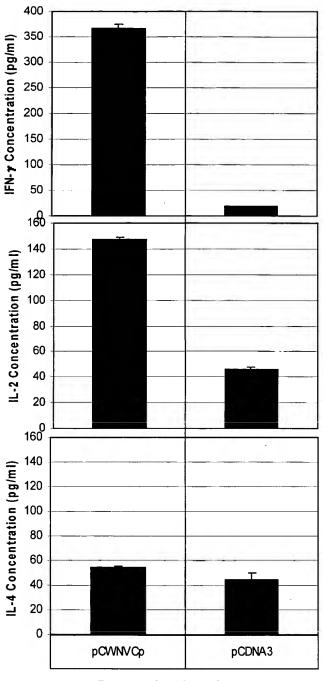


Fig. 12C



Docket Number: UPN-4105 (M2351)
Title: Compositions and Methods of Using Capsid
Protein from Flaviviruses and Pestiviruses
Inventors: David B. Weiner and Joo-Sung Yang
Sheet 19 of 30



Immunization Group

Figure 13

Docket Number: UPN-4105 (M2351)
Title: Compositions and Methods of Using Capsid
Protein from Flaviviruses and Pestiviruses
Inventors: David B. Weiner and Joo-Sung Yang
Sheet 20 of 30

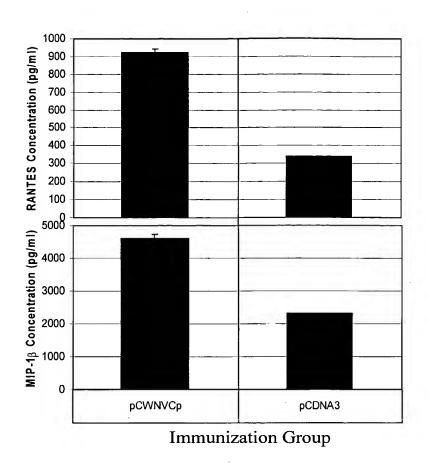


Figure 14

Docket Number: UPN-4105 (M2351)
Title: Compositions and Methods of Using Capsid of the properties of t

Fig. 15A

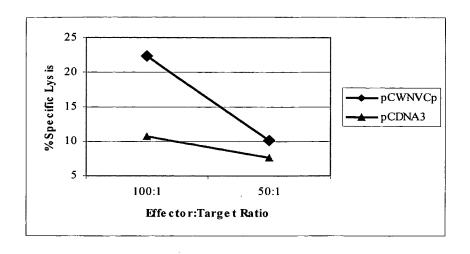
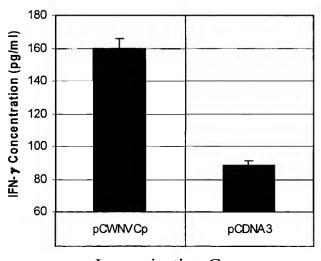


Fig. 15B



Immunization Group

Docket Number: UPN-4105 (M2351)
Title: Compositions and Methods of Using Capsid
Protein from Flaviviruses and Pestiviruses
Inventors: David B. Weiner and Joo-Sung Yang
Sheet 22 of 30

Fig. 16A

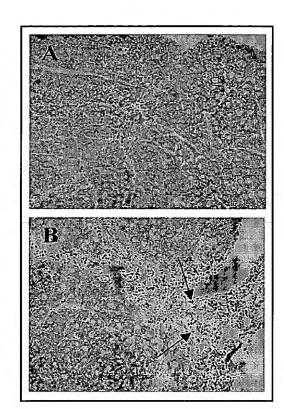
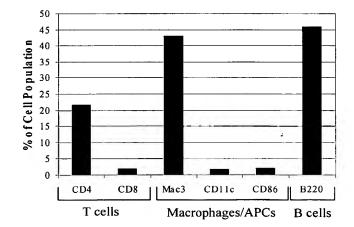


Fig. 16B

Fig. 16C



Docket Number: UPN-4105 (M2351)
Title: Compositions and Methods of Using Capsid
Protein from Flaviviruses and Pestiviruses
Inventors: David B. Weiner and Joo-Sung Yang
Sheet 23 of 30

MSKKPGGPGKSRAVNMLKRGMPRVLSLTGLKRAMLSLIDGRGPTRFVLALLAFFRFTAIAPTRAVLDRWRSVNKQTAMKHLLSFKKELGTLTSAINRRSSKQKKRGGKTGIAFMIGLIAGVGA> ${\tt MSKKPGGPQKSRAVNMLKRGMPRVLSLIGLKRAMLSLIDGKGPIRFVLALLAFFRFTAIAPTRAVLDRWRGVNKQTAMKHLLSFKKELGTLTSAINRRSSKQKKRGGKTGIAVMIGLIASVGA$ 110 90 9 80 80 9 20 40 20 20 WNVCp JEV Cp KJV Cp [899]

MTK KPGGPGKNRA I NMLKRGLPRVFPLVGVKRVVMSLLDGRGPVRFVLAL I TFFK FTALA PTKALLGRWKAVEKSVAMKHLTSFKRELGTLI DAVNKRGRKQNKRGGNEG - SIM>

[446]

WNVCp

MSKKPGGPGKSRAVNMLKRGMPRVLSLIGLKRAMLSLIDGKGPIRFVLALLAPFRFTAIAPTRAVLDRWRGVNKQTAMKHLLSFKKELGTLTSAINRRSSKQKKRGGKTGIAVM

RNTPFNMLKRERNRVSTVQQLTRFSLGMLQGRGPLKLFMALVAFLRFLT1PPTAG1LKRWGT1KKSKAINVLRGFRKEIGRMLNILNRR> KSRAVNMLKRGMPRVLSLIGLKRAMLSLIDGKGPIRFVLALLAFPRFTAIAPTRAVLDRWRGVNKQTAMKHLLSFKKELGTLTSAINRR 20 9 20 40 30 20 DEN2 Cp [220] WNVCp

Figure 17

Docket Number: UPN-4105 (M2351)
Title: Compositions and Methods of Using Capsid
Protein from Flaviviruses and Pestiviruses
Inventors: David B. Weiner and Joo-Sung Yang Sheet 24 of 30

Fig. 18 Alignment of WNVCp protein sequence to other viral proteins

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Search Analysis for Sequence: WNVCaa Search from 1 to 123 where origin = 1 Date: June 15,2001 Time: 19:48:02	s for Sequenc to 123 where 2001	e: WNVCaa origin = 1	W S W	Matrix: pam250 matrix Score Region from 1 to 123 Maximum possible score: 590	50 matrix from 1 to ible score:	123 590					
Database: UserFolder: Alignment-AC6/01	Folder: Align	ment-AC6/01									
	10	20	30	40	20	09	7.0	80	06	100	110

WNVCaa	10 20 SKKPGGPGKSRAVNALKRGAPRVLSLI	20 Mlkrgæprvi	30 LSLIGLKRAM	40 LSLIDGKGPI)	50 Revlallaf e	60 FRFTAIAPTRA	70 ravidrwrgynk <u>o</u> t/	80 AMKHLLSI	90 KKELGTLTSA	90 100 FKKELGTLTSAINRRSSKQKKRGG	110 120 KRGGKTGIAVMIGLIASY	120 IGLIASVGA
1. HIV-1 89. 1. HIV-1 89. [41] WNVCaa	1. HIV-1 89.6 Vpr protein 1. HIV-1 89. [41] WNVCaa						6 TWTGVEA RWRGVNK	60 TWTGVEALIRILQQLLFI HFR-IGCRHSRIGIIQQRRT-RNG>) I-HFR-IGCF KKELGTLTSF	80 HSRIGIIQQE 	90 RRT-RNG> 1	
2. Herpes Sil	2. Herpes Simplex virus major capsid prot	or capsid	protein							N N		
2. HerpesMaj [64] WNVCaa	160 AVKTV-ASI 11 PGKSRAVN	160 170 VKTV-ASALQFGVD-ALEI GKSRAVNMLKRGMPRVLSI	180 ER-GLINTVLS 	190 SVKLRHAPPM SLIDGKGPIR	200 4FILQTLADPT REVLALLAFFR	210 TFTERGFSKTV RFTAIAPTRAV	220 VKSDLIAMFKR V VLDRWRGVNKQ	160 170 180 190 200 210 220 230 240 1 250 260 200 240 1 250 260 240 1 250 260 240 1 250 260 240 1 250 260 240 1 250 260 240 1 250 260 240 1 250 260 240 1 250 240 1 250 260 240 1 250 260 240 1 250 260 240 1 250 260 240 1 250 260 240 1 250 260 240 1 2 260 240 1 2 260 240 1 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2	24 DRAENMGSG 	1 250 0 250 FFSQYSRLSEN 111 1	240 250 260 SGFSQYSRLSEMVAAVSGESVI 	KGV>

ر	LNI.>	_	IGL
300	YAPFARL	=	KTGIAVM
290	LSSLAKHGE	=	SSKOKKRGG
280	CNEVNSFKAA	=	TLTSAINRE
270	HPLARTAKVK	_ = _	KHLĽSFKKELG
260	LOKTERGVRI	_	RGVNKQTAMK
250	LIVKTVLDHI	=======================================	APTRAVLDRW
5 1 240	SNVAQARFSGI	= = =	ALLAFFRFTAI
230	IDANDAVI	= =	(GPIREVL
220	-IHQGMHMVAGF	 = _ _	LKRAMLSLIDGK
. 210	TN-FLIKFLL	= =	GMPRVLSLIG
200	HMMVIFRLMRTN-F	_	KSRAVNMLKRGMPR
3. EBOLANucl	[48]		WNVCaa

3. Ebola nuclear protein

Docket Number: UPN-4105 (M2351)
Title: Compositions and Methods of Using Caps
Protein from Flaviviruses and Pestiviruses
Inventors: David B. Weiner and Joo-Sung Yang Sheet 25 of 30

6. Rubella capsid protein 6. Rubellaca [38 }

WNVCaa

 Ebola glycoprotein
 EbolaGlyc
 39] WNVCaa

120 130 KPDGSECLPAAPDGIRGFPR>

4. Ebola glycoprotein4. EbolaGlyc 120[30] KPDGSEC

WNVCaa

Docket Number: UPN-4105 (M2351)
Title: Compositions and Methods of Using Capsid Protein from Flaviviruses and Pestiviruses
Inventors: David B. Weiner and Joo-Sung Yang
Sheet 26 of 30

Fig. 18 (continued) Alignment of WNVCp protein sequence to proapoptotic proteins

WNVCaa	10 20 30 40 50 60 70 80 90 100 120 120 MSKKPGGPGKSRAVNALKRAMELSLIGLKRAMESLIDGKGPIRFVLALLAFFRFTAIAPTRAVLDRWRGVNKQTAMKHILSFKKELGTLTSAINRRSSKQKKRGGKTGIAVMIGLIASVGA	30 MPRVLSLIGLKRA	40 Meseidgkgpii	50 Revlallaeere	60 FTAIAPTRAVI	70 LDRWRGVNKÇ	80 Q tamk hllsff	90 KELGILISAIN	100 trrsskokku	110 RGCKTGIAVMIC	120 GLIASVGA
1. HuBAK [47]	гинс	170 180 190 200 LHHCIARWIAQRGGWVAALNLGNGPILNVLVVLGVVLLGQEVVRR>	10 1- ALNLGNGPI)	90 200 LNVLVVLGVVLI) .GQFVVRR> 						
WNVCaa	LKRG	LKRGMPRVLS1.IGLKRAMLS1.IDGKGPIRFVLALLAFFRFTAIAPTR	MLSLIDGKGPI	REVLALLAFFRI	FTAIAPTR						
2. huBcl2ass [37]	v					-				TGALLLO	30 TGALLLQGMIAAVDT>
WNVCaa										TGIAVMI	GLIASVGA
							ω –			ы —	
3. HuBIK [37]						Ö	100 1 TEDIRDVLRF	110 FMDGFTTLKENIN	120 IM-REWRSPN	100 110 120 130 140 QTEDIRDVLRFWDGFTTLKENIM-RFWRSPNPGSWVSCQVLLALL>	40 ALL>
WNVCaa						— <u>x</u>		 KKELGTLTSAIN	 NRRSSKQKKI		
4. HuBID [36]							E 2	40 FRRELDALGHEL>	^		
WNVCaa							- E	FKKELGTLTSAI			
5. HuBad [35]	130 DSFKKGLPR>	130 (GLPR>									
WNVCaa	NMLKRGMPR	MPR					•				

Fig. 19 Alignment of HIV-1 89.6 Vpr protein sequence to other viral proteins

Search Analysis for Sequence: HIV-1 89.6 VpraaMatrix: pam250 matrix Search from 1 to 96 where origin = 1 Score Region from 1 to 96 Date: June 15,2001 Time: 19:57:09

Database: UserFolder: Alignment-AC6/01

HIV-1 89.6 MEQAPEDQGPQREPYNDWTLELLEELKNEAVRHFPRIWLHSLGQHIYETYGDTWTGVEALIRILQQLLFIHFRIGCRHSRIGIIQQRRTRNGASKS 9

1. p230 nonstructural protein/ Sindbis virus
1. p230nonst
[50]
FROLDNSRTRO

HIV-1 89.6

West Nile Virus capsid protein
 WNVCaa

Docket Number: UPN-4105 (M2351)
Title: Compositions and Methods of Using Capsid
Protein from Flaviviruses and Pestiviruses Inventors: David B. Weiner and Joo-Sung Yang

Sheet 27 of 30

HIV-1 89.6 45]

3. Cucumovirus 2A protein
3. Cumbermos
[36]

HIV-1 89.6

DEHRESYIRDRRA>

Docket Number: UPN-4105 (M2351)
Title: Compositions and Methods of Using Capsid
Protein from Flaviviruses and Pestiviruses
Inventors: David B. Weiner and Joo-Sung Yang
Sheet 28 of 30

110 EFGNTFSVPDPLR-EVQRL> ||:|| || || || || || TYGDTWTGVEALIRILQQL

4. Cucumovirus 2A protein 4. Cumbermos

HIV-1 89.6

40]

160 WLWSEGQGAVFY> ||| || || || RIWLHSLGQHIY

Rubella virus capsid protein
 Rubellaca
 33 }

6. Nipah virus fusion protein 6. NipahFusi [44]

HIV-1 89.6

.sky.sd...vf-> | | | | | | | | | | IRILQQLLFIHF

HIV-1 89.6

Docket Number: UPN-4105 (M2351)
Title: Compositions and Methods of Using Capsid Protein from Flaviviruses and Pestiviruses Inventors: David B. Weiner and Joo-Sung Yang. Sheet 29 of 30

Fig. 19 (continued) Alignment of HIV-1 89.6 Vpr protein sequence to proapoptotic proteins

90 IIQQRRTRNGASKS	
80 [GCRHSRIG	
70 Qollfiher1	
60 TGVEALIRII	
50 60 70 Gohiyetygdiwigvealirilgollfih	
40 PRIWLHSLGQI	
30 SLKNEAVRHF	
20 INDWTLELLER	
10 20 MEGAPEDQGPQREPYNDWTLEL	
9.68	
HIV-1 89.6 MEQA	

40 50 60 70 801 90 100 PQASIRQSQEEP-EDLRPEIRIGDEFNETYTRRVFADYREAE-DHPQMVI-LQLLRFIF-RL> 11	A N 1 80 40 50 70 80 LRPEIRIGELRRIGDEENETYTRRAFADYREAE-DHPQMVI-LQLLRFIF-RL> 1 1 1 1 1 1 1 1 1 1	90 100 110 120 RNVARQLHIPLQSEPVVTDAFLA-VAGHIFSAG-ITWGKVVSLYSVAAGL> 	V 140 110 120 130 14	140 150 160 WILDFIRERLIGMIQDQ-GGWVRLL>
1. MuBIM	2. RatBOD	3. MuMtd	4. huBc12ass	5. HuBcl-2as [44]
[65]	[53]	[61]	[56]	
HIV-1 89.6	HIV-1 89.6	HIV-1 89.6	HIV-1 89.6	



Docket Number: UPN-4105 (M2351)
Title: Compositions and Methods of Using Capsid
Protein from Flaviviruses and Pestiviruses
Inventors: David B. Weiner and Joo-Sung Yang
Sheet 30 of 30

150 160 VFQSWWDRNLGR> | | | | | | | | HFPRIWLHSLGQ

The state of the s

6. HuBad [36] HIV-1 89.6